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Stretch-independent activation of the mechanosensitive cation channel in oocytes of *Xenopus laevis*

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Abstract

Oocytes of the South African clawed toad *Xenopus laevis* possess in their plasma membrane a so-called stretch-activated cation channel (SAC) which is activated by gently applying positive or negative pressure (stretch) to the membrane patch containing the channels. We show here that this mechanosensitive channel acted as a spontaneously opening, stretch-independent non-selective cation channel (NSCC) in more than half of the oocytes that we investigated. In 55% of cell-attached patches (total number of patches, 58) on 30 oocytes from several different donors, we found NSCC opening events. These currents were increased by elevating the membrane voltage or raising the temperature. NSCC and SAC currents shared some properties regarding the relative conductances of $Na^+ > Li^+ > Ca^{2+}$, gating behaviour and amiloride sensitivity. Stretch-independent currents could be clearly distinguished from stretch induced SAC currents by their voltage and temperature dependence. Open events of NSCC increased strongly when temperature was raised from 21 to 27°C. NSCC currents could be partly inhibited by high concentrations of extracellular Gd^{3+} and amiloride (100 and 500 μM, respectively). We further show exemplarily that NSCC can seriously hamper investigations when oocytes are used for the expression of foreign ion channels. In particular, NSCC complicated investigations on cation channels with small conductance as we demonstrate for a 4 pS epithelial Na^+ channel (ENaC) from guinea pig distal colon. Our studies on NSCCs suggest the involvement of these channels in oocyte temperature response and ion transport regulation. From our results we suggest that NSCC and SAC currents are carried by one protein operating in different modes.

Keywords: *Xenopus laevis* oocyte; Stretch-activated cation channel; Non-selective cation channel; Epithelial Na^+ channel; Patch clamp

1. Introduction

Oocytes of the South African clawed toad *Xenopus laevis* devoid of their follicular enclosure provide a frequently used model system for investigating ion transport processes mediated by channels, transporters and ion pumps. Beside the 'classical' ion conductances, reviewed by Dascal in 1987 [26], particularly in the last three years, formerly unknown endogenous transport systems of the oocyte emerged. It has been shown that native oocytes possess amiloride-sensitive Na^+ conductances [1], Na^+/Ca^{2+} exchangers [2], monovalent cation channels blocked by extracellular Ca^{2+} [3,4] as well as Ca^{2+}-inactivated Cl^- channels [5–7] and non-selective cation channels activated by maitotoxin [8].

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In addition to these recently revealed endogenous ion conductances, *Xenopus* oocytes own mechanosensitive channels which were also investigated in prokaryotes, eukaryotes and in tissues of plants and animals [9,10]. They respond to moderate membrane stress by changes in open probability and hence were termed stretch-activated (SA) channels which can conduct cations or anions and sometimes exhibit non-selective properties [9]. The plasma membrane of *Xenopus* oocytes is endowed with a stretch-activated cation channel (SAC) which is one of the most extensively described endogenous ion channels of *Xenopus* oocytes [11–14]. In cell-attached and excised patches, SAC was activated by applying moderate suction or pressure to the pipette. It could be shown that these channels were sensitive to high concentrations of amiloride ($K_{0.5} \approx 500$ μM) [15] and that complete block of SAC could be achieved by low concentrations of Gd^{3+} (10 μM) [16].

Here we report a stretch-independent mode of SAC activity which could be described as NSCC at physiological oocyte membrane potentials (V_m). Elevation of voltage or temperature increased strongly the number of NSCC open events. The channel exhibited mechanosensitivity induced by means of positive or negative applied pressure. In contrast to previous reported high sensitivity of SAC for Gd^{3+}, NSCC activity was blocked efficiently only by high concentrations of Gd^{3+} (100 μM). We demonstrate that NSCC can cause disturbances when oocytes are used for the expression of low conducting ion channels. Our data suggest a physiological role of NSCC in ion homeostasis due to the activity of this channel at oocyte membrane resting potential. Parts of the results have been presented at the spring meeting of the German Physiological Society and were published in abstract form in Pflügers Arch. Eur. J. Physiol. 433 (1997) R154.

2. Materials and methods

2.1. Oocytes and RNA preparation

Female *X. laevis* were purchased from the African *Xenopus* Facility (Knysna, Republic of South Africa). Defolliculated oocytes were obtained as described in detail earlier [17]. After shrinking by hyperosmolarity (100 mM NaCl+400 mM mannitol) oocytes were devitellinated mechanically with fine forceps. Protocols used for preparation and injection of mRNA were identical with those reported in detail previously [18,19].

2.2. Solutions

NaCl, and LiCl pipette solutions (100 mM) contained Ca^{2+} (1 mM) as the only divalent cation. Investigations for testing the Ca^{2+} conductance were made with $CaCl_2$ (100 mM). HEPES (5 mM) was applied as pH buffer and *N*-methyl-D-glucamine (NMDG) was used to set the pH to 7.2 NMDG-Cl (100 mM) and supplemented with Ca^{2+} (100 μM) as probe for a possible Cl^- conductance of the NSCC. Bath solutions contained a sodium Ringer (NaCl, 90 mM). During measurements of reversal potentials of heterologously expressed epithelial Na^+ channels, oocytes were depolarised with a high potassium Ringer (K^+, 90 mM; Na^+, 10 mM). Investigations with Gd^{3+} were made with the high dissolvable salt gadoliniumchloride ($GdCl_3$). Therefore, we could be sure that the exceedingly stable Gd^{3+} was active in

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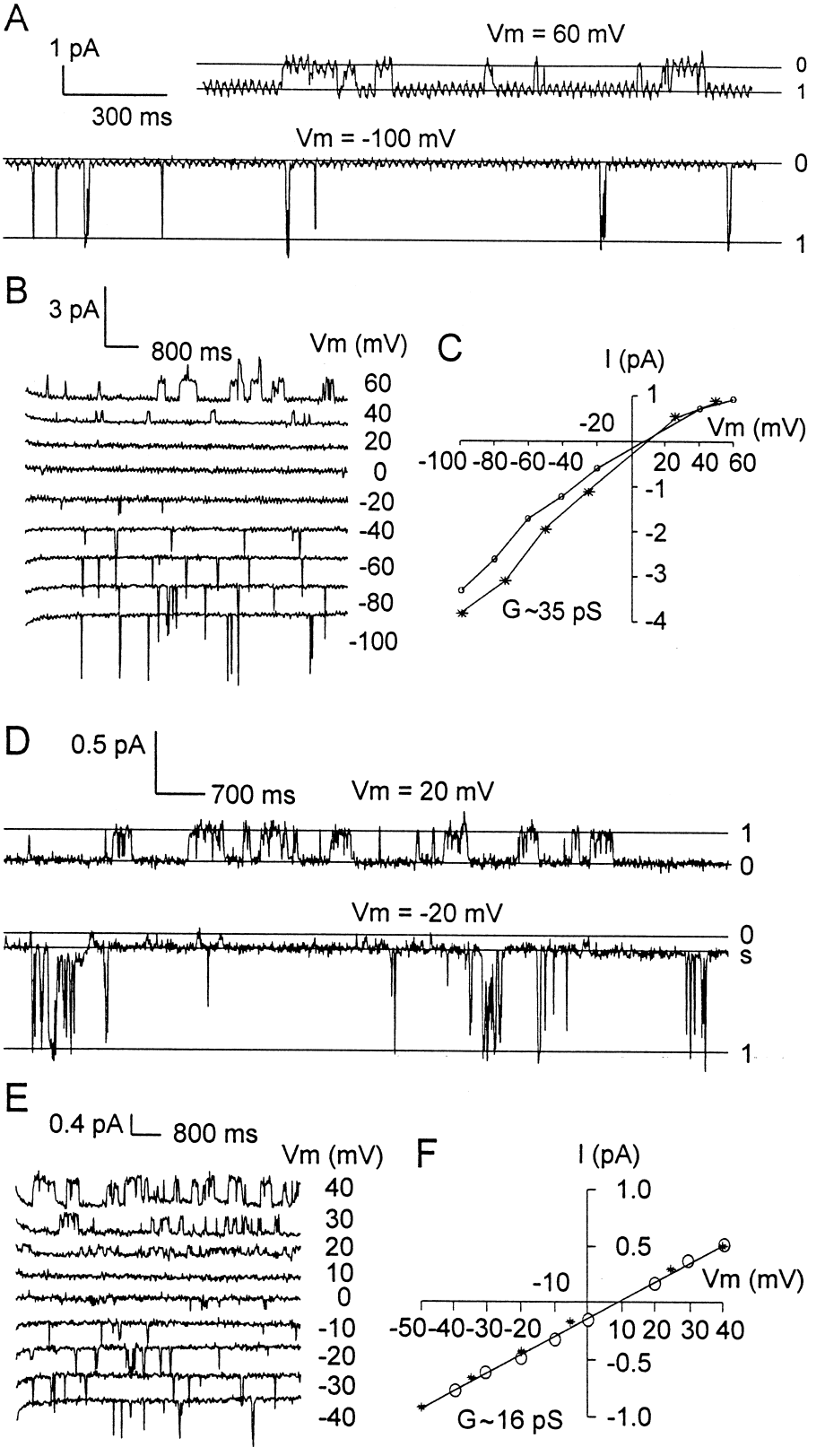
Fig. 1. Voltage dependence of NSCC currents recorded in the cell-attached configuration. Measurements were made with NaCl (100 mM) or LiCl (100 mM) in the pipette and Na^+ Ringer (90 mM) in the bath. (A) Recordings of NSCC outward currents at 60 mV and inward currents at −100 mV. Outward currents had long open events and inward currents appeared as spikes. The ground line (0) and the main open state (1) are marked by lines. (B) NSCC currents evoked by voltage pulses with a duration of 8 s show the change from long openings ($V_m = 60$ and 40 mV) to short openings ($V_m = -20$ to −100 mV). At least two channels were present in the patch. (C) Current–voltage relationships (*I–V* curves) of NSCC (non-suction induced = open circles; suction-induced = asterisks) reflect a slope conductance of about 35 pS. (D) Recordings of NSCC currents with Li^+ (100 mM) in the pipette. The main open states (1) at 20 and −20 mV and a slow gating sublevel (s) at −20 mV are marked by lines. (E) NSCC currents recorded during voltages pulses with a duration of 8 s. (F) *I–V* curves obtained from voltage pulse experiments without (open circles) and with suction (asterisks). Slope conductance of NSCC is about 16 pS.


A
1 pA
300 ms
Vm = 60 mV
0
1
Vm = -100 mV
0
1
B
3 pA
800 ms
Vm (mV)
60
40
20
0
-20
-40
-60
-80
-100
C
I (pA)
1
-20
Vm (mV)
-100 -80 -60 -40
20 40 60
-1
-2
-3
-4
G~35 pS
D
0.5 pA
700 ms
Vm = 20 mV
1
0
Vm = -20 mV
0
s
1
E
0.4 pA
800 ms
Vm (mV)
40
30
20
10
0
-10
-20
-30
-40
F
I (pA)
1.0
0.5
-10
Vm (mV)
-50-40-30-20
10 20 30 40
-0.5
-1.0
G~16 pS

our pipette solutions. Substances were purchased from Sigma (Deisenhofen, Germany).

2.3. Electrophysiology

Borosilicate capillaries with a wall-thickness of 0.16 mm from Hilgenberg (Malsfeld, Germany) were pulled with a two stage puller PP-83 (Narishige, Tokyo, Japan). Pipette resistances were in the range of 4–7 MΩ. Patch-clamp measurements were performed in the attached and inside-out configuration using a LM-PC patch-clamp amplifier (List Medical, Darmstadt, Germany).

Data acquisition and analysis were done with pClamp 5.5 (Axon Instruments, Foster City, USA). Square pulse experiments were done with the pClamp-Clampex routine and directly stored on hard disk. Continuous single-channel measurements were stored on a modified conventional DAT recorder (AIWA, Tokyo, Japan). Subsequent signal low-pass filtering was accomplished with an 8-pole Bessel filter series 902 (Frequency Devices, Haverhill, USA). Data were digitised with at least four times f_{Bessel}. Open probabilities (P_o) were calculated as described elsewhere [20].

Membrane voltage (V_m) of attached patches refer to the oocyte resting potential (V_r) minus the applied pipette clamp potential (V_p). V_r was measured at the end of the experiment by penetrating the oocyte membrane with the patch pipette and immediately analysed in the current clamp mode. Due to decline of V_r during the patch-clamp investigations V_r was rounded to −15, −20, −25 mV, e.g. $V_r = -18.5$ mV was measured and accounted as −20 mV. Flow of cations into the cytosol is conventionally termed inward current and plotted downward in all graphs. Unless otherwise stated, data refer to at least three different patches obtained from different oocytes and donors.

2.4. Temperature control

An electric coil surrounded the bath chamber (about 0.5 ml test solution volume) and was mounted in a Petri dish (35 mm diameter). The oocyte within the test solution was gently heated by the coil which was connected to a DC power supply. The actual temperature of the bath was measured by a conventional electrical thermometer whose thermoresistor was placed near the oocyte (distance of the resistor to the oocyte, < 5 mm). Heating the bath solution from 20 to 27°C could be accomplished within 5 min and cooling back to initial values was sometimes accelerated by cooling the coil.

2.5. Pressure application

Hydrostatic pressure was used for seal formation and for suction-induced activation of the NSCC. Accuracy of pressure adjustment was in the range of 5 mm H_2O column.

3. Results

To examine whether the mechanosensitive NSCC exhibited measurable influence on ion permeability of the oocyte plasma membrane in a stretch-independent way, we investigated the non-stretch activated openings of these channels. In a series of 58 experiments, we found in 32 non-stretched cell-attached patches NSCC open events, indicating well-defined pressure-independence of this channel. Fig. 1 depicts representative non-stretched induced NSCC currents. Recordings were made with NaCl or LiCl in the pipette and reflected an open probability (P_o) up to 0.02 at negative V_m.

3.1. Voltage dependence

Membrane voltage strongly influenced the gating properties of NSCC currents. Inward currents had short open states (spikes) with mean open times below 4 ms, whereas outward currents tended to longer open states with mean open times of about 10 ms (Fig. 1A,D). The open probability (P_o) of NSCC inward currents was about 0.02 ($V_m = -100$ mV), whereas outward currents exhibited a $P_o > 0.15$ ($V_m = 60$ mV) at 24°C. At membrane potentials below 10 mV, NSCC mediated flow of cations (Na^+ or Li^+) into the oocyte. Positive V_m (> 10 mV) caused a cation efflux from the oocyte cytoplasm to the outside, which tended to repolarise the patch. Therefore, under physiological conditions (i.e. about −60 mV),

NSCC mediates small Na^+ and Ca^{2+} inward currents thereby contributing to the oocyte membrane potential.

Fig. 1B and E show NSCC currents in the range from −100 to 60 mV and reflect the change in gating behaviour when raising V_m from negative to positive potentials. The main open state of NSCC was used for the construction of current–voltage (*I–V*) curves (Fig. 1C,F). *I–V* curves constructed from measurements with NaCl (100 mM) in the pipette exhibited a moderate inward rectification (Fig. 1C) whereas *I–V* relationships with LiCl (100 mM) were nearly linear (Fig. 1F).

3.2. Selectivity of the NSCC conductance

We investigated the conductive properties of the NSCC for Na^+, Li^+, Ca^{2+} and NMDG-Cl. Fig. 1 shows NSCC currents with Na^+ and Li^+ (both 100 mM) in the patch-pipette solution. As demonstrated in Fig. 1C,F the slope conductances were 35 pS for Na^+ ($-100 < V_m > -20$) and 16 pS for

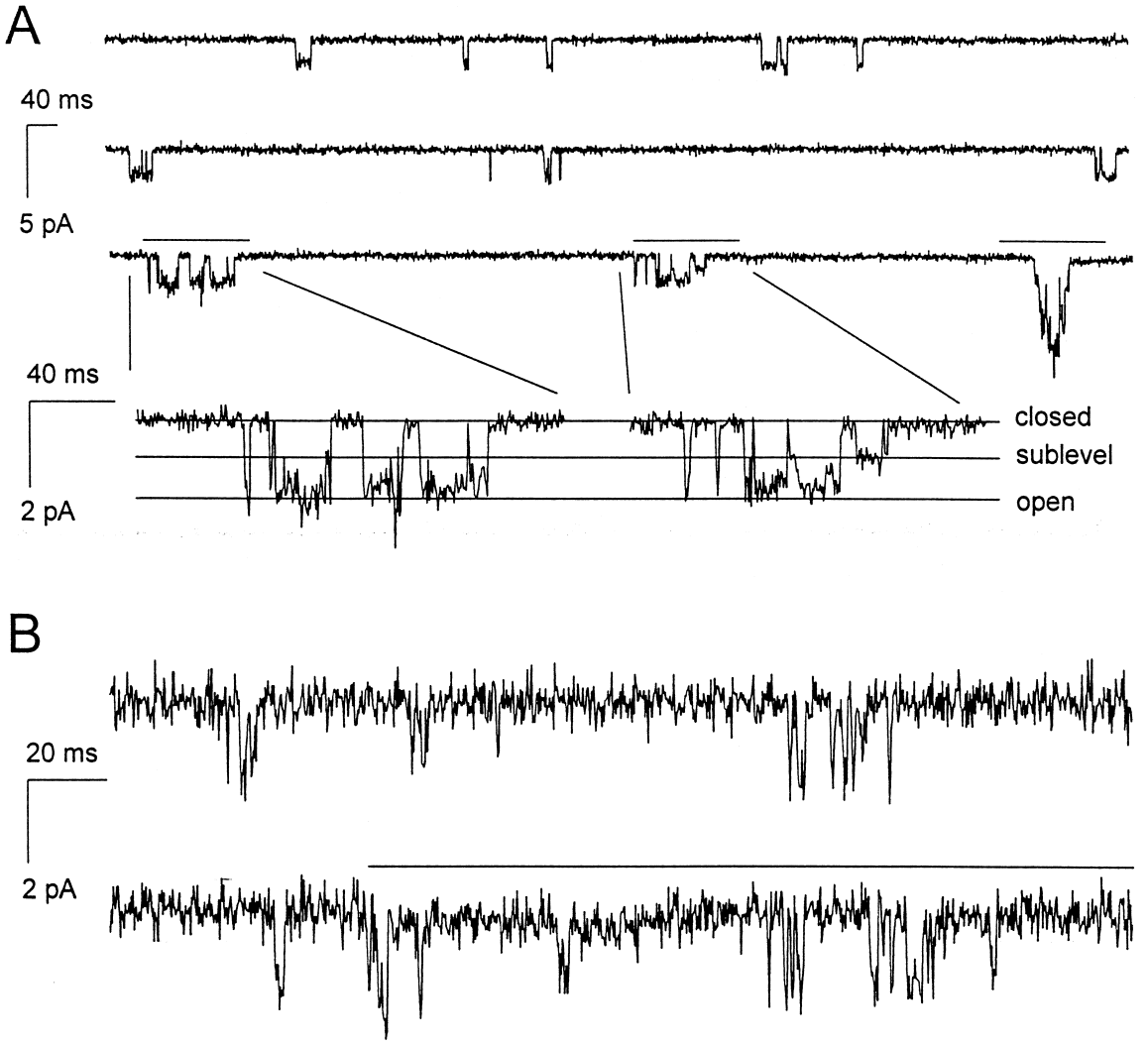


Fig. 2. Sodium and calcium conductances of the NSCC measured in the cell-attached mode. (A) Pressure-free and suction-induced openings of NSCC conducting Na^+ (90 mM in the pipette). During gentle suction (marked by lines) a sublevel beside the main open state was visible. Measurements were made with $V_m = -60$ mV at 21°C. Up to 4 NSCC could be activated by suction. The last current trace shows the open states in detail. (B) Suction-free and suction-induced NSCC currents conducting Ca^{2+} recorded at $V_m = -90$ mV and 26.2°C. The pipette solution contained $CaCl_2$ (100 mM). The period of suction is marked by a line. $CaCl_2$ induced a noisy ground line and flickery open events of NSCC with mean open times below 0.5 ms.

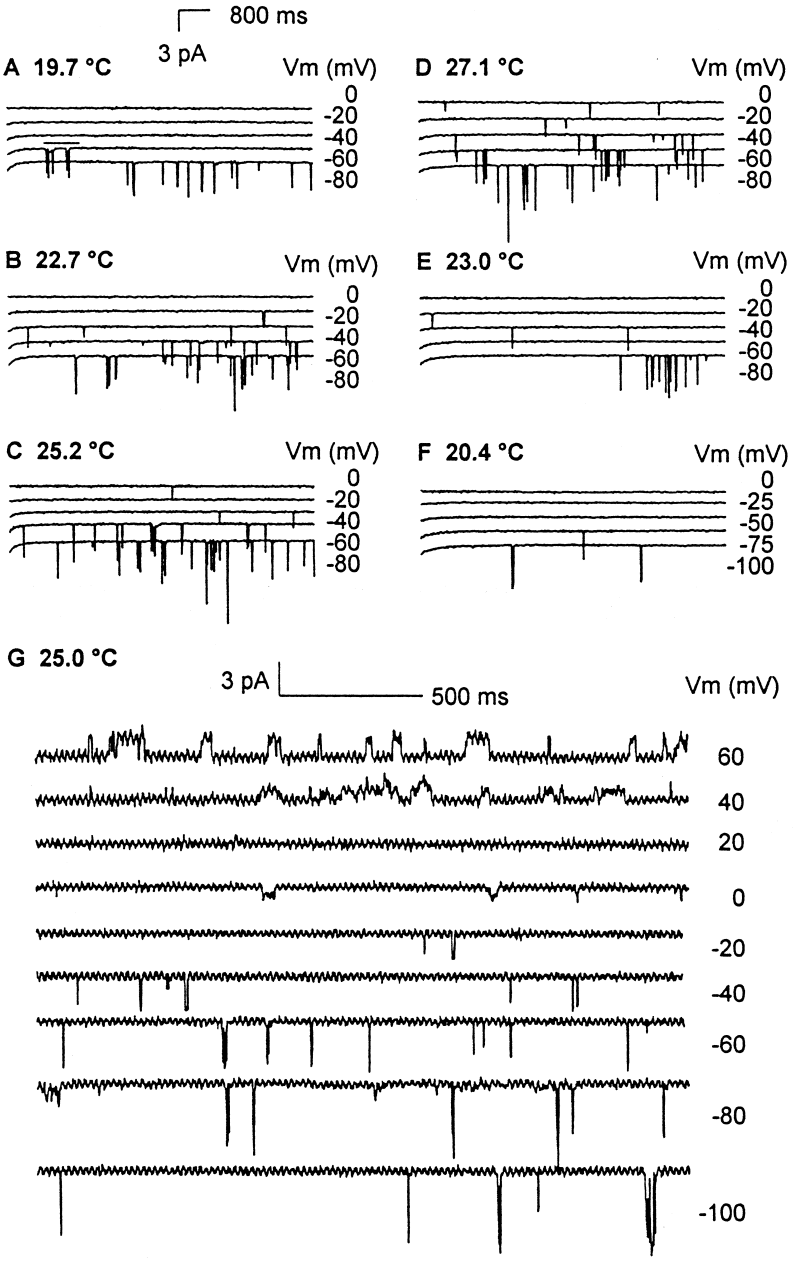


Fig. 3. Temperature dependence of NSCC currents recorded in the cell-attached mode. (A) NSCC currents were recorded during voltage pulses with a duration of 8 s. The initial temperature was 19.7°C. Gentle suction (about 4 cm H_2O column) was applied at −60 mV. The period of suction is marked by a line. (B–D) The number of NSCC open events increased during heating. Up to four channels were simultaneously active. (E,F) Cooling off the bath solution reduced the number of NSCC open events to below initial values. Shown are data from a patch with NaCl (100 mM) in the pipette and Na^+ Ringer (90 mM) in the bath. (G) Samples of NSCC outward and inward currents at 25°C recorded with an enhanced sampling rate (1 ms).

Li^+ ($-40 < V_m > 0$). The Ca^{2+} conductance is shown in Fig. 2B. $CaCl_2$ (100 mM) in the pipette solution caused noisy background currents and flickery open states of NSCC inward currents with open time constants below 0.5 ms. The slope conductance for Ca^{2+} was about 15 pS in the rage $-50 < V_m > -90$. The sequences of relative slope conductances were Na^+ (1.0), Li^+ and Ca^{2+} (about 0.5). The remarkable

Ca^{2+} conductivity of the NSCC argues for a physiological role in Ca^{2+} homeostasis of the oocytes.

During applications of negative V_m, no patch currents could be detected with an NMDG-Cl (100 mM) solution (supplemented with Ca^{2+}, 100 μM) in the pipette indicating that the NSCC was nearly impermeable for Cl^- and the large cation $NMDG^+$. Only strong suction (20 cm H_2O column) elicited small $NMDG^+$ currents via the NSCC (data not shown).

NSCC was sensitive to externally applied pressure. *I–V* curves derived from NSCC currents during the application of suction (2–10 cm H_2O column) and without applied pressure exhibited similar shapes and reflected single-channel slope conductances of about 35 pS with Na^+ and 16 pS with Li^+ (Fig. 1C,F).

Subconductance levels of NSCC currents could be detected in patches with and without applied pressure (Figs. 1D and 2A). NSCC currents had at least two sublevels beside the flickery main open state. As exemplarily shown in Fig. 1D, sublevels could have slower kinetics than the main open state.

3.3. Influence of temperature

Fig. 1 shows voltage-dependent open events of NSCC at constant temperatures. Elevation of bath temperatures revealed a strong sensitivity of the NSCC on temperature. To further investigate this dependence we performed experiments with short time square voltage pulses (2 or 8 s) and long time (up to 10 min) constant holding voltages under varying bath temperatures. Fig. 3A–F shows inward currents derived from square pulse experiments measured on a single patch by raising the temperature from 20 to 27°C and subsequently cooling back to 20°C. When temperatures exceeded 25°C (Fig. 3B,C) up to four channels were simultaneous open at $V_m = -80$ mV. Comparison of current traces in Fig. 3A at −80 mV with current traces in Fig. 3F at −75 and −100 mV indicate that this temperature-induced activation of NSCC was only partly reversible. A negative pressure impulse of 1 s duration (marked by a line in Fig. 3A) was applied during the recording of the current trace at −60 mV, exhibiting the mechanosensitivity of the NSCC. Fig. 3G

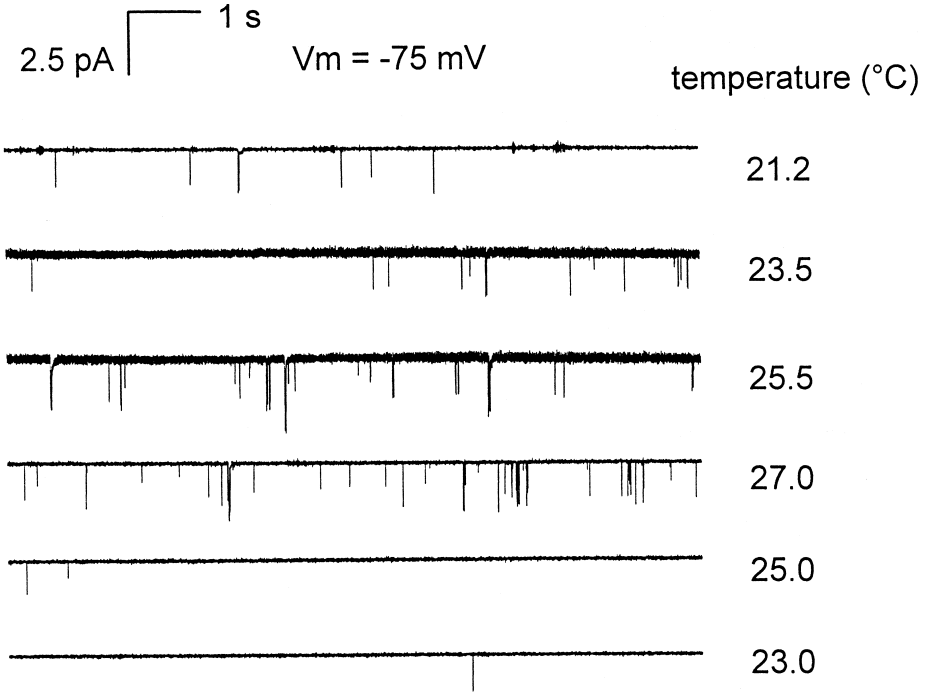


Fig. 4. Dependence of NSCC open events on temperature during long-time recordings. Shown are data from an cell-attached patch with NaCl (100 mM) in the pipette. Heating the bath solution from 21.2 to 27.0°C increased the number of open events about 6-fold. Cooling off the bath temperature revealed that NSCC activation by temperature was only partly reversible.

shows in more detail the NSCC outward and inward currents which were recorded during square pulse experiments.

The experiment shown in Fig. 4 was performed with a constant holding voltage (−75 mV) and demonstrates the increase of NSCC open events during a heating period of the bath chamber (6 events at 21°C, 13 events at 23.5°C, 27 events at 25.5°C and 35 events at 27.0°C). Deactivation of NSCC appeared during cooling down. Only three open events within 20 s could be detected within the last two current recordings in Fig. 4.

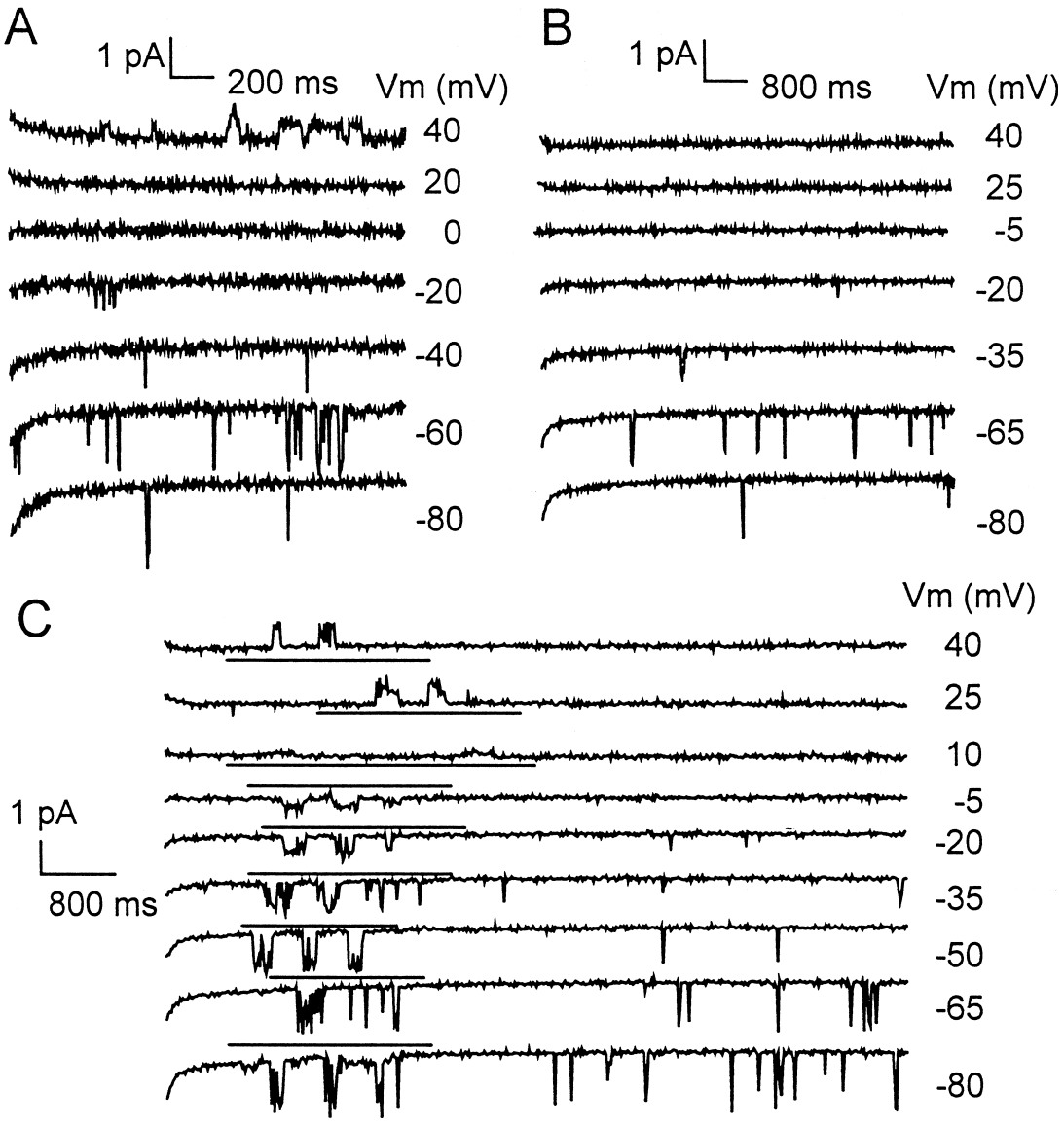


Fig. 5. Gd^{3+} (100 μM) reduced NSCC currents in attached patches. The pipette solution contained LiCl (100 mM), the temperature was in the range of 24–25°C. (A) Typical non-inhibited NSCC currents recorded during voltage pulses with a duration of 2 s. (B) Recordings during voltage pulses with a duration of 8 s. Gd^{3+} (100 μM) in the pipette solution inhibited NSCC currents and reduced the number of open events about 90% compared to non-inhibited NSCC activity. (C) Current traces of the mechanosensitive NSCC recorded from the same patch as shown in B, yet with periods of applied suction (4–8 cm H_2O column). Periods during the application of suction are marked by lines.

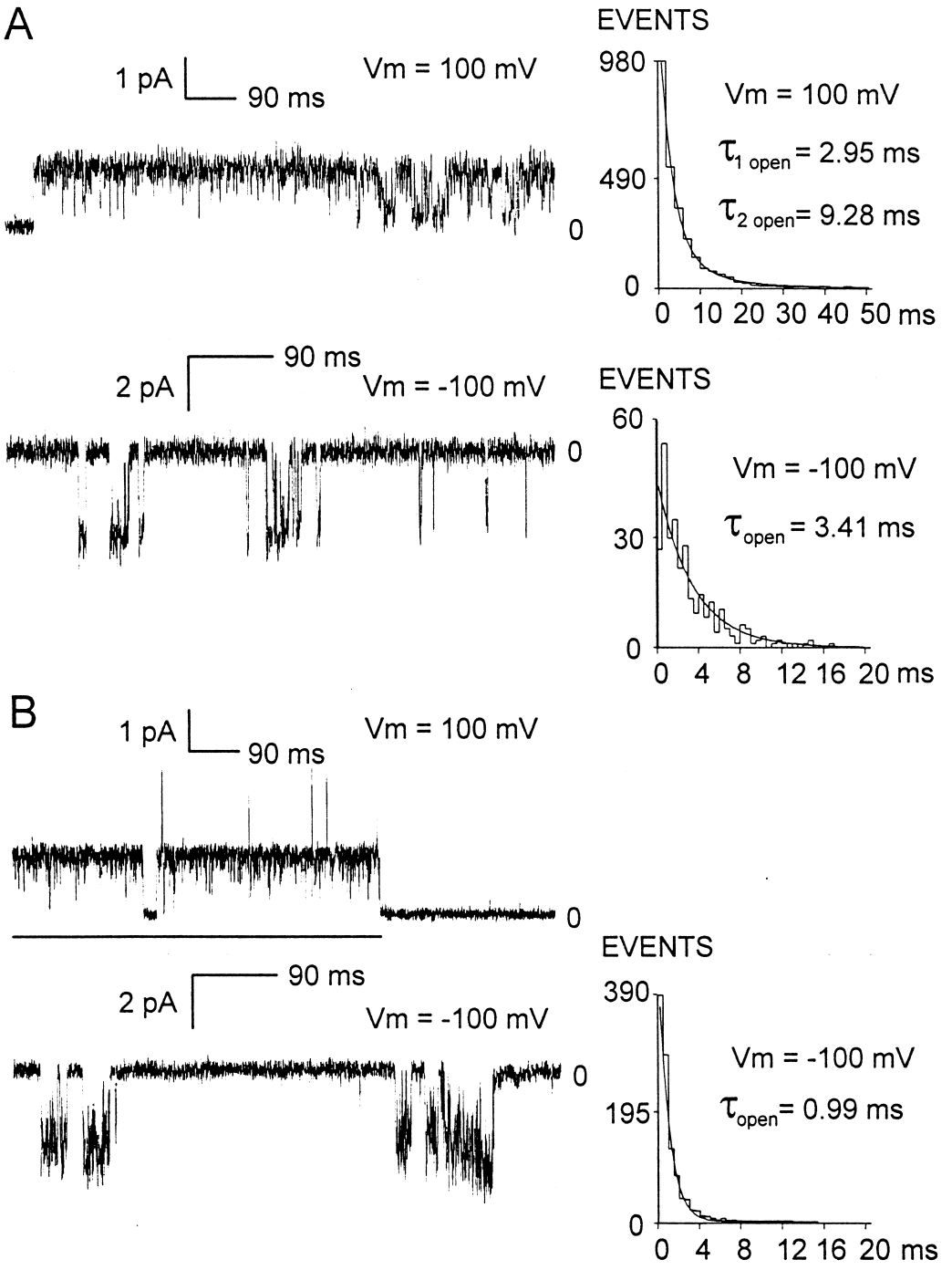


Fig. 6. Amiloride block of NSCC measured in the cell-attached configuration. Pipette solution contained NaCl (100 mM), distributions reflect intra-burst open events. (A) Non-inhibited NSCC outward and inward currents. The open time distribution of outward currents at $V_m = 100$ mV could be fitted with a short (2.95 ms) and a long (9.28) time constant. The distribution of inward currents at $V_m = -100$ mV could be sufficiently fitted by one time constant of 3.4 ms. (B) Amiloride (500 μM) in the pipette solution suppressed NSCC outward currents. Outward currents could be only induced by strong negative pressure (marked by a line). Inward currents appeared to be pressure-independent and exhibited a fast flickery block with an open time constant of about 1 ms.

3.4. Inhibition by gadolinium

Gadolinium (Gd^{3+}) is the most commonly used inhibitor for investigating the physiological role of mechanogated ion channels [9,21]. Complete block of the oocyte SAC was reported on outside-out patches with 10 μM extracellular Gd^{3+} [16]. We found that Gd^{3+} in this concentration was unable to reduce effectively NSCC openings in the attached configuration. As shown in Fig. 5A, many NSCC open events could be seen at temperatures quite normal in our laboratory during summer time (i.e. at 24°C). Higher concentrations of Gd^{3+} (100 μM) in the pipette solution reduced the number of NSCC open events by about 90%. Under these conditions, current traces in Fig. 5B exhibited only 15 open events within 5.6 s, whereas current traces recorded with 10 μM Gd^{3+} in the pipette showed 34 openings within 1.4 s (Fig. 5A).

Open events of NSCC could be enhanced by application of positive or negative pressures (2–15 cm H_2O column) and reduced the inhibitory effect of Gd^{3+}. During the record of current traces in Fig. 5C short suction periods (2–4 cm H_2O column) were applied to the patch (marked with lines in Fig. 5C). The currents after suction application were similar to NSCC currents of non-stretched patches as shown in Fig. 5B. Suction-induced NSCC currents could only be blocked completely by using 250 μM Gd^{3+} in the pipette solution.

3.5. Amiloride block

Previous studies on SAC under stretch-applied conditions revealed that high amiloride concentrations (500 μM) induced a 50% block of this channel in *Xenopus* oocytes [10]. Non-suction induced NSCC currents showed similar low sensitivity for amiloride. Fig. 6A demonstrates the gating behaviour of non-inhibited NSCC. Inward currents ($V_m = -100$ mV) reflected an open time constant of about 3 ms. A strong depolarisation of the patch ($V_m = 100$ mV) elicited outward openings with two time constants (about 3 and 9 ms) and open probabilities up to 0.5. Visible effects on NSCC currents required an amiloride concentration of 500 μM in the pipette medium (Fig. 7B). This amiloride concentration produced enlarged burst times on inward currents at $V_m = -100$ mV) and a fast flickery block which reduced the conductance. The open time constant decreased from 3.4 ms (non-inhibited NSCC) to about 1 ms with amiloride (500 μM). With amiloride (500 μM) in the pipette, no outward currents could be detected when depolarising the patch with $V_m = 100$ mV. To induce amiloride-inhibitable outward currents of the mechanosensitive NSCC, currents were activated by applying suction to the pipette. The first record of Fig. 7B shows such an amiloride-inhibited NSCC outward current elicited by suction (8 cm H_2O column). The period with suction is marked by a line.

3.6. NSCC interferences with expressed Na^+ channels

NSCC openings complicated the discrimination between endogenous and expressed channels, especially in the case of similar conductances and gating properties [11]. Disturbances caused by NSCC were examined in our laboratory during expression experiments with epithelial Na^+ channels (ENaC) from guinea pig distal colon. These channels exhibited a low conductance (4 pS) and were highly amiloride sensitive ($K_{0.5} \approx 80$ nM). To amplify ENaC currents, we used patch pipettes with relatively large diameter having low resistances of about 4 MΩ. Consequently, these patches additionally contained many NSCCs (up to 10). Non-inhibited NSCC currents in some cases made investigations of the expressed ENaC nearly impossible. Therefore, experiments were performed with Gd^{3+} in the pipette solutions. Fig. 7 demonstrates ENaC openings from patches on mRNA-injected oocytes with Li^+ (100 mM) as conducting cation and Gd^{3+} (100 μM) as NSCC blocker in the pipette. Nevertheless, NSCC currents sometimes superimposed expressed ENaC currents (Fig. 7A). When ENaC was inhibited by low doses of amiloride, the open probability of this channel decreased from $P_o = 0.8$ (non-inhibited) to $P_o < 0.05$ (amiloride, 0.5 μM) and mean open times were reduced from seconds to milliseconds (Fig. 7B). Under such conditions ENaC and NSCC open events appeared as spikes which could only be distinguished by their main open current state. Fig. 7B also shows an NSCC sublevel (marked with an arrow) which occurred only when the channel was conducting Li^+.

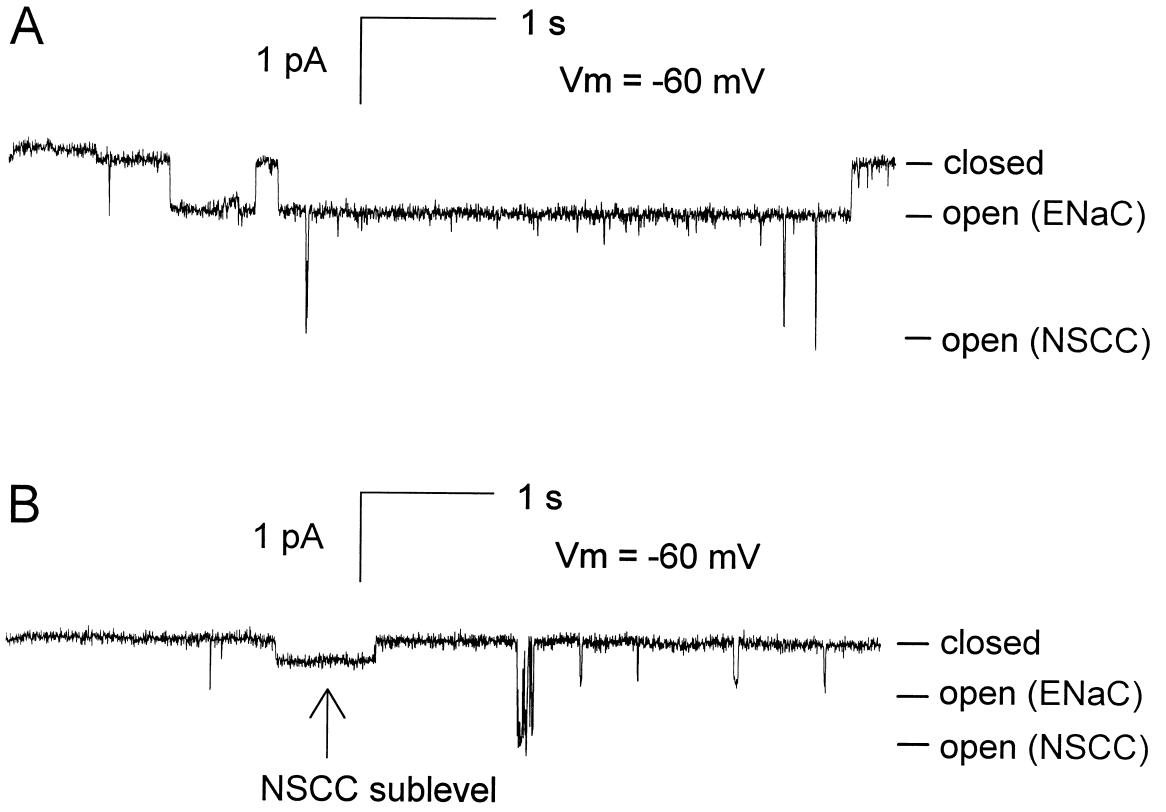


Fig. 7. NSCC interfered with expressed ENaC. Test solutions contained LiCl (100 mM) with Gd^{3+} (100 μM) in the pipette and a high K^+ Ringer (90 mM) in the bath. (A) Non-inhibited ENaC currents with an open time in the range of seconds were superimposed from Gd^{3+}-inhibited NSCC open events. Main open conductance states of the ENaC and NSCC are marked beside the current trace. (B) Amiloride (0.5 μM) inhibited ENaC had mean open times in the millisecond range very similar to the mean open time of NSCC. ENaC and NSCC main open conductance states are marked beside the current trace. A NSCC sublevel is indicated by an arrow and can pretend an ENaC open event.

4. Discussion

Although the endogenous stretch-activated cation channel (SAC) in oocytes of *X. laevis* has been investigated thoroughly by many working groups, its properties as non-stretch activated, non-selective cation channel has been only poorly analysed [10,11,13]. While the functional roles of SAC in oocytes has not been clearly established, an increasing number of studies support the view of physiological significance of these mechanosensitive channels in regulating cell volume by sensing membrane tension [22,23]. Ca^{2+} conductance of stretch-activated cation channels was supposed to interfere with signal cascades regulated by Ca^{2+} [16].

4.1. Influence of pressure

The present study focuses on the properties of an NSCC. In the majority of our experiments, after seal formation, no pressure was applied to the patch pipette except of short periods to analyse the number of mechanosensitive NSCCs in the patch. It could be argued that a patched membrane might be permanently stretched due to its geometric form which develops from negative pressure thereby promoting seal formation. Can the observed NSCC activity arise from this artificial form of a patch (patch-intrinsic pressure)? Two observations emerging from our studies strongly argue against NSCC activation from patch-intrinsic pressure: (1) the less negative pressure for seal formation was applied (sometimes only 1 cm H_2O column) the more NSCC activity could be measured; and (2) NSCC responded with

enhanced openings during changes of pressure. Continuously applied pressure caused a decline of NSCC currents down to non-stretched patch currents. This adaptation on continuous pressure also impaired the sensitivity to voltage and temperature. In most patches, persisting negative pressure (e.g. 10 cm H_2O column) caused a complete deactivation of NSCC currents within minutes. Corresponding observations were described for the SAC [14]. From our observations we conclude that the described results are due to NSCC properties which are an intrinsic feature of SACs in *Xenopus* oocytes.

Another question may arise from small residual pressures on the patch-pipette solution. Yang and Sachs [13] investigated SAC without externally applied pressure and with positive and negative pressures (1, 2 and 3 cm Hg). They found that SAC was active without pressure ($P_o < 0.1$) and minimum of P_o was measured with a positive pressure of 1 cm Hg (about 13 cm H_2O column), which seems very high. We found that NSCC currents could be transiently minimised (but did not disappear) with positive pressures in the range of 0–2 cm H_2O. Therefore we conclude that our results can be attributed to non-pressure-activated NSCC.

All these considerations cannot exclude the possibility of artificial channel activities induced by using the patch-clamp technique. Furthermore, it could be argued that a pipette tip, which encloses a patch, could cause residual pressure and could be an artificial condition for a cell membrane which might generally modulate conductances.

4.2. Channel density and conductance

Like endogenous Cl^- channels of the oocyte, which were shown to be dependent on external and intracellular Ca^{2+}, namely the Ca^{2+}-inactivated Cl^- channel [5–7] and Ca^{2+}-activated Cl^- channels [24,25], NSCCs are abundant in similar high plasma membrane densities of about one million molecules per oocyte [11]. This high channel density should enable NSCC to provoke significant whole-cell currents. Indeed, integrated NSCC inward currents over a period of 2 s could have average currents up to 0.05 pA at $V_m = -80$ mV (data not shown). The corresponding whole oocyte inward current generated by NSCCs can amount to a maximum of 50 nA and could be easily masked by other electrogenic transport systems endogenous in oocytes [26]. Depolarising voltages applied to the whole oocyte are known to induce a whole orchestra of Cl^- and K^+ currents which could seriously hamper the adequate analysis of whole cell NSCC outward currents. Consequently, outward currents of the whole oocyte should be investigated in presence and absence of high concentrations of Gd^{3+} (250 μM) to resolve the NSCC outward current.

While Methfessel et al. [11] reported an oocyte endogenous cation channel of 25 pS which exhibited similar gating behaviour and sublevel open states, we found a conductance of about 35 pS with NaCl (100 mM) and Ca^{2+} (1 mM) in the pipette (Fig. 1). Presumably, Methfessel et al. [11] used higher concentrations of divalent cations in their pipette media which reportedly reduce inward currents of the so-called SAC [12,16]. We found NSCC currents without applied stretch (see Fig. 1). The voltage dependence of these currents resemble those of currents recorded under stretched conditions reported by Taglietti and Toselli [12].

4.3. Temperature dependence

Our results demonstrate that temperature-induced activation of NSCC was only partly reversible and accompanied by deactivation. Temperature is a well known modulator of transport rates [13]. These authors estimated a temperature coefficient (Q10) of 2.2 for SAC open times at 22 and 26°C but did not analyse the enormous increase of open events induced by those temperature changes. Another temperature-dependent non-selective cation current in oocytes was induced by depolarisation and was strongly increased by temperature elevation from 21 to 26°C. In this study, it could be further shown that *Xenopus* connexin38 was involved in the generation of this current [27]. An expressed Cl^- current (i.e. ClC-0) in *Xenopus* oocytes was reportedly inhibited by increased temperatures [28].

4.4. Gating properties and selectivity

Membrane voltage strongly influenced NSCC gating behaviour. Outward currents had rather long open times, whereas the main inward open state ap-

peared with short open times (spikes). Beside this, NSCC exhibited at least three sublevels. Fig. 1 shows the slow gating properties of a sublevel measured with Li^+ in the pipette which is comparable with the gating behaviour of epithelial Na^+ channels expressed in oocytes [19]. Methfessel et al. [11] investigated SAC gating with the noise analysis at −70 mV and found two Lorentzian time constants (2 and 18 ms) reflecting a complex gating behaviour of the NSCC. The sublevel shown in Fig. 1D might produce corner frequencies below 5 Hz due to the slow gating of NSCC and a fast value produced by spiky main open currents.

SAC inward currents reportedly carry Ca^{2+} [16] and had a selectivity sequence of $Na^+ > Li^+ > Ca^{2+}$. Ca^{2+} can enter the oocyte through NSCCs which exhibit a single channel conductance of about 15 pS in the range of $-90\ mV < V_m < -50\ mV$. This Ca^{2+} conductance could modulate the intracellular Ca^{2+} concentration and influence endogenous Ca^{2+} channels or other Ca^{2+}-dependent ion channels. Recently, Jeffrey et al. [29] described the voltage and temperature dependence of cardiac Ca^{2+} channels expressed in *Xenopus* oocytes. They measured Ba^{2+} inward currents at positive V_m, which might have been unintentionally superimposed by NSCC outward currents which could have modulated the shape and shift of their measured *I–V* curves.

4.5. Sensitivity to Gd^{3+} and amiloride

Clearly, the presence of NSCC currents interfere with measurements of currents through expressed Ca^{2+} channels or Ca^{2+}-dependent ion channels. To prevent unwanted influences of NSCCs on expressed currents, it would be desirable to block endogenous NSCC. Gd^{3+} is exceedingly stable in this oxidation state and was described as a potent blocker of SAC in *Xenopus* oocytes [30]: only 10 μM of Gd^{3+} reportedly should cause complete and reversible block of SAC in outside-out patches [16]. Since this patch configuration has severe impacts on the cytoskeleton [14] and the inside-out configuration led to a fast inactivation of NSCC currents, we restricted ourselves to cell-attached measurements. In contrast to Yang and Sachs [16], we needed, in this configuration, much higher concentrations of Gd^{3+} (up to 250 μM) for effective inhibition of suction-activated NSCC (Fig. 5C). Therefore, if using Gd^{3+} as inhibitor for NSCCs, one should be aware that only high concentrations (> 200 μM) could guarantee effective block of almost all NSCC currents in oocytes. Unfortunately, such high doses of Gd^{3+} could have a lot of side effects on endogenous or expressed conductances (e.g. Ca^{2+} channels). Therefore, the optimal blocker for oocyte NSCC remains to be found.

High concentrations of external amiloride induced a fast flickery block of NSCC inward currents thereby shortening the intra-burst open time constants from 3.4 ms (non-inhibited) to values of about 1 ms. Nevertheless, burst durations of inward NSCC currents appeared prolonged compared with non-inhibited currents (Fig. 6). Similar amiloride concentrations were reported for inhibition of SACs in oocytes [10].

4.6. Interaction with expressed epithelial Na^+ channels

Patch-clamp experiments on epithelial Na^+ channels from guinea pig distal colon expressed in oocytes were seriously disturbed by non-inhibited NSCCs. To characterise the guinea pig ENaC, we performed most of the experiments in the presence of 100 μM Gd^{3+} (Fig. 7). Fortunately, ENaC currents were shown to be unaffected in open probability by Gd^{3+}, but there is evidence that Gd^{3+} might prolong the open time constant. Even with Gd^{3+} (100 μM), NSCC openings hampered the analysis of expressed ENaC blocked by amiloride (0.5 μM). Under such conditions, NSCC and ENaC exhibited open events as spikes which were only discernible by their different main open states (Fig. 7B). Small concentrations of amiloride (0.5 μM) reduced the open probability of guinea pig ENaC from 0.8 (non-inhibited) to values below 0.05, but had no visible influence on NSCC.

Taken together, the plasma membrane of *X. laevis* oocytes contains mechanosensitive non-selective cation channels which can switch from an inactive to a conducting state without the need of externally applied pressure. One protein is responsible for NSCC and SAC currents operating either in a stretch-dependent or a stretch-independent mode. Our studies point to a new mechanistic understanding of the so-called 'stretch activated cation channel' which was

shown to be responsive to voltage and temperature. The relatively poor affinity of NSCC for Gd^{3+} and the high sensitivity to temperature might give new implications to two electrode (whole oocyte) measurements. We further propose that SAC in future should have a second meaning: stretch-independent, non-selective cation channel (NSCC).

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References

[1] W.-M. Weber, K.M. Liebold, W. Clauss, Biochim. Biophys. Acta 1239 (1995) 201–206.
[2] T. Schlief, S.H. Heinemann, J. Physiol. (Lond.) 486 (1995) 124–130.
[3] R.O. Arellano, R.M. Woodward, R. Miledi, J. Physiol. (Lond.) 484 (1995) 593–604.
[4] Y. Zhang, D.W. McBride, O.P. Hamill, J. Physiol. (Lond.) 508 (1998) 763–776.
[5] W.-M. Weber, K.M. Liebold, F.W. Reifarth, U. Uhr, W. Clauss, Pflügers Arch. Eur. J. Physiol. 429 (1995) 820–824.
[6] W.-M. Weber, K.M. Liebold, F.W. Reifarth, W. Clauss, J. Membr. Biol. 148 (1995) 263–275.
[7] F.W. Reifarth, S. Amasheh, W. Clauss, W.-M. Weber, J. Membr. Biol. 155 (1997) 95–104.
[8] A. Bielfeld-Ackermann, C. Range, C. Korbmacher, Pflügers Arch. Eur. J. Physiol. 436 (1998) 329–337.
[9] H. Sackin, in: S.G. Schultz, T.E. Andreoli, A.M. Brown, D.M. Fambrough, J.F. Hoffman, M.J. Welsh (Eds.), Molecular Biology of Membrane Transport Disorders, Plenum Press, New York, 1996, pp. 201–222.
[10] O.P. Hamill, D.W. McBride, Pharmacol. Rev. 48 (1996) 231–252.
[11] C. Methfessel, V. Witzemann, T. Takahashi, M. Mishina, S. Numa, B. Sakmann, Pflügers Arch. Eur. J. Physiol. 407 (1986) 577–588.
[12] V. Taglietti, M. Toselli, J. Physiol. (Lond.) 407 (1988) 311–328.
[13] X.-C. Yang, F. Sachs, J. Physiol. (Lond.) 431 (1990) 103–122.
[14] O.P. Hamill, D.W. McBride, Proc. Natl. Acad. Sci. USA 89 (1992) 7462–7466.
[15] J.W. Lane, D.W. McBride, O.P. Hamill, J. Physiol. (Lond.) 441 (1991) 347–366.
[16] X.-C. Yang, F. Sachs, Science 243 (1989) 1068–1071.
[17] W.-M. Weber, W. Schwarz, H. Passow, J. Membr. Biol. 111 (1990) 93–102.
[18] W.-M. Weber, B. Püschel, J. Steffgen, H. Koepsell, W. Schwarz, Biochim. Biophys. Acta 1063 (1991) 73–80.
[19] K.M. Liebold, F.W. Reifarth, W. Clauss, W.-M. Weber, Pflügers Arch. Eur. J. Physiol. 431 (1996) 913–922.
[20] F.W. Reifarth, T. Weiser, F.-W. Bentrup, Biochim. Biophys. Acta 1192 (1994) 79–87.
[21] R.A. Caldwell, H.F. Clemo, C.M. Baumgarten, Am. J. Physiol. 275 (1998) C619–C621.
[22] J.W. Lane, D.W. McBride, O.P. Hamill, Br. J. Pharmacol. 106 (1992) 283–286.
[23] W. Schütt, H. Sackin, Pflügers Arch. Eur. J. Physiol. 433 (1997) 368–375.
[24] R. Miledi, I. Parker, J. Physiol. (Lond.) 357 (1984) 173–183.
[25] G.P.H. Young, J.D.E. Young, A.K. Deshpande, M. Goldstein, S.S. Koide, Z.A. Cohn, Proc. Natl. Acad. Sci. USA 81 (1984) 5155–5159.
[26] N. Dascal, Crit. Rev. Biochem. 22 (1987) 317–387.
[27] L. Ebihara, Biophys. J. 71 (1996) 742–748.
[28] M. Pusch, U. Ludewig, T.J. Jentsch, J. Gen. Physiol. 106 (1996) 105–116.
[29] T. Jeffrey, A. Allen, G. Mikala, Pflügers Arch. Eur. J. Physiol. 436 (1998) 238–247.
[30] W. Stühmer, Methods Enzymol. 207 (1992) 319–339.